mined as a quantitative measure of the antigen present. A fluorimetric method of antigen immunoassay is disclosed by Eiji Ishikawa in an article entitled, "Enzyme Immunoassay of Insulin by Fluorimetry of the Insulin-glucoamylase Complex," *J. Biochem.*, V. 73, No. 6, 1319–1321 (1973). In this article, immunoassay methods may be applied using fluorimetric sensors as opposed to the traditionally accepted radionuclide methods which are expensive, and also sometimes hazardous when not properly conducted. The article also discloses another method for the chemical linking of an antigen to an enzyme.

A flow-through enzymatic reactor is disclosed in U.S. Pat. No. 3,838,011 wherein enzymatically reactive substances are contained in an aqueous solution, and are quantitatively determined by introducing a sample into a stream which flows at a constant velocity through an enzyme reactor and then through a detector system. Similarly U.S. Pat. No. 4,039,652 discloses a flow system which comprises essentially a syringe filled with immobilized antigen or antibody, wherein the non-immobilized binding partner is placed over the immobilized partner in a supernatant liquid and after a sufficient reaction time has passed, the fluid is drained from the syringe. The eluted fluid is subjected to a determination of the relative amount of the labeled compound retained or eluted from the column.

SUMMARY OF THE INVENTION

An object of this invention is to provide a method and apparatus for the quantitative determination of an antigen in biochemical fluids.

Another object of this invention is to provide a method and apparatus for the rapid quantitative determination of an antigen contained in a sample biochemical fluid by passing the sample through a series of sequential stages wherein the antigen reacts with reagents to obtain a measurable product which is quantitative index of the concentration of the antigen in the sample.

A further object of this invention is to provide an immobilized antibody, reactable with an antigen in a biochemical sample, which is saturated with an enzyme-antigen complex, wherein the antigen in the sample reacts with the immobilized antibody-complex to produce an equilibrium state between the antigen in the sample and the antigen complex whereby an amount of the complex is released into the sample and can be measured to give a quantitative index of the concentration of the antigen originally in the sample.

Another object of this invention is to provide a method and apparatus for the quantitative determination of an antigen in a biochemical sample wherein the sample is injected into a buffered flowing aqueous stream which is sequentially contacted and reacted in a series of stages to produce a measurable product which is detected and measured in a detection stage to give a quantitative determination of the concentration of the antigen in the original sample.

An additional object of the invention is to provide an apparatus which allows the rapid, low cost analysis of antigens in biochemical samples without the necessity of discarding valuable antibodies after each analysis.

The above and other objects of the invention are achieved by a method and apparatus which provides for the rapid flow-through determination of antigens in biochemical fluids using a flow-through system design. The system comprises immobilized reagents which are stable for long periods of time and are not discarded as is the case with present heterogeneous and homogeneous antigen assay procedures. The invention produces a quantitative measure of the quantity of antigen in a fluid sample by passing the sample through a solubilization stage, wherein an antigen-enzyme complex is reversibly bound to an immobilized antibody. The complex is liberated into the flowing stream by competitive equilibrium with the antigen in the unknown sample. The sample flows from the solubilization stage to a conversion stage, wherein the antigen-enzyme complex is caused to react with a substrate of the enzyme to thereby form a measurable product. The measurable product then flows to a detection stage comprising detection means which senses the concentration of the measurable product in the flowing stream. The detection means generates a signal which is communicated to readout means which quantitatively represents the concentration of the antigen in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

Other objects and advantages of the invention will become readily manifest to those skilled in the art from a reading of the following detailed description of the invention when considered with the accompanying drawings in which:

FIG. **1** is a schematic flow diagram according to the method and apparatus of the invention with a solubilization, conversion and detection stage;

FIG. **2** is a schematic flow diagram essentially similar to FIG. **1** including in addition, a second buffer supply and an alternative embodiment of the conversion stage.

FIG. **3** is a schematic flow diagram essentially similar to FIG. **1** disclosing a second alternative embodiment of the conversion stage.

DETAILED DESCRIPTION OF THE INVENTION

The method and apparatus according to the present invention provides for the quantitative determination of an antigen in biochemical fluids, particularly in human body fluids. The invention generally comprises a unique three stage flow-through system comprising solubilization, conversion and detection stages. In the solubilization stage, an immobilized antibody specific to the antigen to be determined, is saturated with an enzyme-labelled antigen, wherein the antigen is the same as the antigen to be determined. The sample containing an unknown quantity of the antigen is introduced into a flowing stream of buffered solution which carries it into the solubilization stage. Therein the antigen in the sample displaces by competitive binding the labelled antigen, and labelled antigen enters into the flowing stream. The stream carries the labelled antigen into the conversion stage wherein the enzyme is allowed to react with a substrate in a single or multistep reaction process to produce a measurable product. The product is measured in the detection stage.

The invention will be better understood by reference to FIG. **1**. Reservoir **20** contains buffer solution which is pumped by pump **22** to provide a flowing buffer stream. The stream enters injection port **24** which provides means to mix the sample with the buffer stream in the mixing zone defined by the injection port **24**. Various antigens contained in biochemical fluids are commonly monitored, e.g. natural compounds such as insulin, and synthetic compounds such as drugs, i.e. phenobarbital and the like. The stream and sample flow into solubilization stage **28**, which comprises an antibody,